

The risk for HEV infection through transfusions of donated blood emerged in West Africa in a similar way as described in European countries. Further assessment of the transfusion risk associated with HEV-positive donors will require an evaluation of HEV RNA in prospective donors and posttransfusion surveillance of occurrence of hepatitis.

Acknowledgments

We thank the nurses and staff of the National Blood Transfusion Center, Ouagadougou.

K.A.T. received funding from the 3rd-cycle university scholarship program of the Embassy of France in Burkina Faso (<http://www.burkina.campusfrance.org>) and a University Paris-Sud XI co-tutelle fellowship.

References

1. Rerambiah LK, Rerambiah LE, Bengone C, Djoba Siawaya JF. The risk of transfusion-transmitted viral infections at the Gabonese National Blood Transfusion Centre. *Blood Transfus.* 2014;12:330–3. <http://dx.doi.org/10.2450/2013.0144-13>
2. Stramer SL, Moritz ED, Foster GA, Ong E, Linnen JM, Hogema BM, et al. Hepatitis E virus: seroprevalence and frequency of viral RNA detection among US blood donors. *Transfusion* 2016;56: 481–488. <http://dx.doi.org/10.1111/trf.13355>
3. Ehteram H, Ramezani A, Eslamifard A, Sofian M, Banifazl M, Ghassemi S, et al. Seroprevalence of Hepatitis E virus infection among volunteer blood donors in central province of Iran in 2012. *Iran J Microbiol.* 2013;5:172–6.
4. Sauleda S, Ong E, Bes M, Janssen A, Cory R, Babizki M, et al. Seroprevalence of hepatitis E virus (HEV) and detection of HEV RNA with a transcription-mediated amplification assay in blood donors from Catalonia (Spain). *Transfusion.* 2015;55:972–9. <http://dx.doi.org/10.1111/trf.12929>
5. Cheng XF, Wen YF, Zhu M, Zhan SW, Zheng JX, Dong C, et al. Serological and molecular study of hepatitis E virus among illegal blood donors. *World J Gastroenterol.* 2012;18:986–90. <http://dx.doi.org/10.3748/wjg.v18.i9.986>
6. Gallian P, Lhomme S, Piquet Y, Saune K, Abravanel F, Assal A, et al. Hepatitis E virus infections in blood donors, France. *Emerg Infect Dis.* 2014;20:1914–7. <http://dx.doi.org/10.3201/eid2011.140516>
7. Hewitt PE, Ijaz S, Brailsford SR, Brett R, Dicks S, Haywood B, et al. Hepatitis E virus in blood components: a prevalence and transmission study in southeast England. *Lancet.* 2014;384:1766–73. [http://dx.doi.org/10.1016/S0140-6736\(14\)61034-5](http://dx.doi.org/10.1016/S0140-6736(14)61034-5)
8. Traoré KA, Rouamba H, Nebie Y, Sanou M, Traoré AS, Barro N, et al. Seroprevalence of fecal-oral transmitted hepatitis A and E virus antibodies in Burkina Faso. *PLoS ONE.* 2012;7:e48125. <http://dx.doi.org/10.1371/journal.pone.0048125>
9. Taha TE, Rusie LK, Labrique A, Nyirenda M, Soko D, Kamanga M, et al. Seroprevalence for hepatitis E and other viral hepatitis among diverse populations, Malawi. *Emerg Infect Dis.* 2015;21:1174–82. <http://dx.doi.org/10.3201/eid2107.141748>
10. Traoré KA, Ouoba JB, Huot N, Rogée S, Dumarest M, Traoré AS, et al. Hepatitis E virus exposure is increased in pork butchers from Burkina Faso. *Am J Trop Med Hyg.* 2015;93:1356–9. <http://dx.doi.org/10.4269/ajtmh.15-0321>

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Porcine Deltacoronavirus, Thailand, 2015

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DOI: <http://dx.doi.org/10.3201/eid2204.151852>

To the Editor: Porcine deltacoronavirus (PDCoV) was first reported in Hong Kong in 2012 and included the HKU15-44 and HKU15-155 strains (1). In early 2014, PDCoV was reported in pigs with diarrhea on swine farms in Ohio, USA (2), and later in other states (2–5). In April 2014, PDCoV strain KNU14-04 was reported in pigs in South Korea (6). A retrospective study in 2012 reported PDCoV strain S27 in Sichuan, China (7). Recently PDCoV strain CNJXN12 has been reported in pigs with diarrhea in Jiangxi, China (8).

There are currently 28 complete PDCoV genomes from China, South Korea, and the United States available in GenBank. We report emergence of PDCoV infections on a commercial swine farm in Thailand.

In June 2015, we investigated reports of acute diarrhea in piglets, gilts, and sows on a swine farm. An outbreak occurred on a commercial swine farm (3,000 sows) located in the eastern province of Thailand. Clinical signs, including acute watery diarrhea, loss of appetite, and agalactia, were observed in gilts and sows in the breeding and gestation houses. Subsequently, piglets in farrowing houses had clinical signs (depression, fever, watery diarrhea, and severe dehydration). Although clinical signs were detected less frequently in fattening pigs in growth-finishing houses, PDCoVs were later detected from blood samples of fattening pigs.

The outbreak lasted 6 weeks (June 10–July 20, 2015). The mortality rate was 27.63% (829/3,000) in sows and 64.27% (2,892/4,500) in piglets but was lower than that usually observed for porcine epidemic diarrhea virus (PEDV) infection. A total of 865 (19.22%) piglets died and were culled during 10 production weeks. Postmortem examination of dead piglets showed emaciated animals and yellow pasty feces. Intestines and colons showed thin walls with a watery content and curdled milk. Histopathologic examination showed shortened and fused villi in the jejunum and ileum. An attenuated and vacuolated cytoplasm in enterocytes was also observed (online Technical Appendix Figure 1, <http://wwwnc.cdc.gov/EID/article/22/4/15-1852-Techapp1.pdf>) (9,10).

We examined 30 samples from the affected swine farm. Blood ($n = 10$), intestine ($n = 8$), lymph node ($n = 2$), feces ($n = 6$), and feed ($n = 4$) samples were collected for 2 day-old piglets and 17-, 19-, and 20-week-old fattening pigs. A total of 26 samples were positive for PDCoV by reverse transcription PCR (2) (online Technical Appendix Table 1). Because sick pigs had clinical signs similar to those of pigs with other swine virus diseases, all samples were tested for transmissible gastroenteritis coronavirus; PEDV; rotaviruses A, B, and C; porcine reproductive and respiratory syndrome virus; and circovirus. All test results were negative.

We selected 2 PDCoVs (S5011 and S5015L) for whole-genome sequencing and 14 PDCoVs for sequencing of spike (S), envelope (E), membrane (M), and nucleocapsid (N) genes and the 3'-untranslated region (UTR). Nucleotide sequences obtained were submitted to GenBank (online Technical Appendix Table 2).

Sequence analysis of the 2 PDCoVs from Thailand showed that their whole genomes had 99.98% nt identity (only 4 nt differences) with each other and highest nucleotide identities with PDCoVs from China (98.43% with AH2004). S gene sequences showed greatest diversity (99.97%–100% nt identities and 99.91%–100% aa identities) for PDCoVs from Thailand and 95.93%–96.68% with other reference PDCoVs, which is consistent with findings of previous report (5). In contrast, E, M, and N genes were conserved (100% nt identities for PDCoVs from Thailand and 99.19%–100% for E genes, 98.28%–99.07% for M genes, and 96.88%–97.81% for N genes with reference PDCoVs) (online Technical Appendix Table 3).

Phylogenetic analysis of the whole genome of PDCoVs from Thailand showed close relatedness with AH-2004, HKU15-44, S27-2012, and HKU15-155 virus strains from China. However, these viruses from Thailand were in a different subcluster than PDCoVs from the United States (Figure; online Technical Appendix Figure 2). PDCoVs identified in this study might represent a new variant of PDCoV because these 2 viruses have unique sequence characteristics: 3-nt (TCT) and 1-nt (A) deletions in the 5'-UTR, 6-nt (AGTTTG) and 9-nt (GAGCCAGTC) deletions in open reading frame 1a/b, and 4-nt (CTCT) insertion in the 3'-UTR (online Technical Appendix Table 4).

We identified PDCoV on a commercial swine farm in Thailand. Affected pigs had clinical signs of acute watery diarrhea, similar to those of pigs infected with PEDV, and had moderate illness and low mortality rates. PDCoVs were detected in symptomatic piglets, sows, and fattening pigs, although clinical signs in fattening pigs were least severe.

Swine farmers and veterinarians should be aware of PDCoV as another causative agent of watery diarrhea in pigs. Similar to PEDV, Wang et al. reported that sequence deletions, insertions, and mutations in PDCoVs in pigs might contribute to variant virus virulence (2).

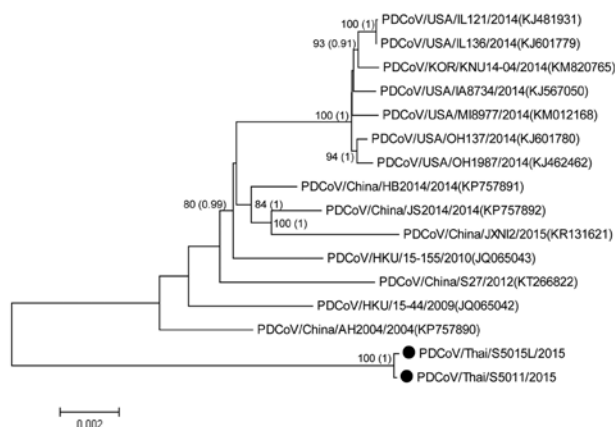


Figure. Phylogenetic analysis of whole-genome sequences of porcine deltacoronaviruses (PDCoVs), Thailand. Black circles indicate strains isolated in this study. The tree was constructed by using MEGA version 6.06 (<http://www.megasoftware.net/>) with the neighbor-joining algorithm and bootstrap analysis with 1,000 replications and BEAST (<http://beast.bio.ed.ac.uk/>) with Bayesian Markov chain Monte Carlo analysis of 5,000,000 generations and an average SD of split frequencies <0.05. Numbers along branches are bootstrap values (posterior probabilities). Scale bar indicates nucleotide substitutions per site.

Our findings might assist in development of diagnostic assays for differentiating PDCoVs in Thailand from PDCoVs in other countries. Because PDCoVs from Thailand were highly related to each other, PDCoV might have transmitted into Thailand by a single event. However, verification of this possibility would be difficult. Similar to the situation in the United States, PDCoV might be underdiagnosed in Thailand.

This study was supported by Chulalongkorn University. R.G. J. (PHD/0026/2556), and A.A. (Senior Scholar Award RTA5780006) were supported by the Thailand Research Fund. The Center of Excellence for Emerging and Re-emerging Infectious Diseases in Animals is supported by Chulalongkorn University.

References

- Woo PC, Lau SK, Lam CS, Lau CC, Tsang AK, Lau JH, et al. Discovery of seven novel mammalian and avian coronaviruses in the genus deltacoronavirus supports bat coronaviruses as the gene source of alphacoronavirus and betacoronavirus and avian coronaviruses as the gene source of gammacoronavirus and deltacoronavirus. *J Virol*. 2012;86:3995–4008. <http://dx.doi.org/10.1128/JVI.06540-11>
- Wang L, Byrum B, Zhang Y. Detection and genetic characterization of deltacoronavirus in pigs, Ohio, USA, 2014. *Emerg Infect Dis*. 2014;20:1227–30. <http://dx.doi.org/10.3201/eid2007.140296>
- Li G, Chen Q, Harmon KM, Yoon KJ, Schwartz KJ, Hoogland MJ, et al. Full-length genome sequence of porcine deltacoronavirus strain USA/IA/2014/8734. *Genome Announc*. 2014;2:e00278–14. <http://dx.doi.org/10.1128/genomeA.00278-14>
- Marthaler D, Jiang Y, Collins J, Rossow K. Complete genome sequence of strain SDCV/USA/Illinois121/2014, a porcine

- deltacoronavirus from the United States. *Genome Announc.* 2014;2:e00218–14. <http://dx.doi.org/10.1128/genomeA.00218-14>
5. Marthaler D, Raymond L, Jiang Y, Collins J, Rossow K, Rovira A. Rapid detection, complete genome sequencing, and phylogenetic analysis of porcine deltacoronavirus. *Emerg Infect Dis.* 2014;20:1347–50. <http://dx.doi.org/10.3201/eid2008.140526>
 6. Lee S, Lee C. Complete genome characterization of Korean porcine deltacoronavirus strain KOR/KNU14–04/2014. *Genome Announc.* 2014;2:e01191–14. <http://dx.doi.org/10.1128/genomeA.01191-14>
 7. Wang YW, Yue H, Fang W, Huang YW. Complete genome sequence of porcine deltacoronavirus strain CH/Sichuan/S27/2012 from mainland China. *Genome Announc.* 2015;3:e00945–15.
 8. Song D, Zhou X, Peng Q, Chen Y, Zhang F, Huang T, et al. Newly emerged porcine deltacoronavirus associated with diarrhoea in swine in China: identification, prevalence and full-length genome sequence analysis. *Transbound Emerg Dis.* 2015;62:575–80. <http://dx.doi.org/10.1111/tbed.12399>
 9. Dong N, Fang L, Zeng S, Sun Q, Chen H, Xiao S. Porcine deltacoronavirus in mainland China. *Emerg Infect Dis.* 2015;21:2254–5. <http://dx.doi.org/10.3201/eid2112.150283>
 10. Jung K, Hu H, Eyerly B, Lu Z, Chepngeno J, Saif LJ. Pathogenicity of 2 porcine deltacoronavirus in gnotobiotic pigs. *Emerg Infect Dis.* 2015;21:650–4. <http://dx.doi.org/10.3201/eid2104.141859>

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Ebola Virus in Breast Milk in an Ebola Virus–Positive Mother with Twin Babies, Guinea, 2015

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DOI: <http://dx.doi.org/10.3201/eid2204.151880>

To the Editor: Field clinicians working during the unprecedented Ebola virus disease (EVD) outbreak in West Africa, which began in December 2013, have been

confronted with complex situations concerning mothers and breast-fed children in which one or both in a pair have tested positive for Ebola virus (EBOV) (1). More data, especially regarding virus shedding in breast milk, is critical to provide better care and guidance in future outbreaks. We report the case of a lactating EBOV-positive mother and her twin babies. The case is anonymously reported with the mother's consent. The study met the Médecins Sans Frontières Ethics Review Board and approved criteria for studies of routinely collected data.

In Guinea in 2015, a woman and her 4-month-old twins (baby 1 and 2) were registered as contacts after the woman's mother tested positive for EBOV (postmortem diagnosis by reverse transcription PCR [RT-PCR]). The woman and her babies, who were exclusively breast-fed, were followed daily by contact tracers. When baby 1 became febrile, the woman left her home to seek help from a traditional healer, bringing both twins with her. A few days later, baby 1 died and was buried without EBOV testing; according to the World Health Organization case definition, baby 1 was a probable EVD case-patient (2).

Eleven days after baby 1 died, the woman became sick; 5 days later, she was admitted to an Ebola treatment center. At admission (day 0), she had headache, loss of appetite, abdominal pain, joint pain, dysphagia, conjunctival injection, and myalgia but was afebrile. On day 1, a blood sample from the woman was positive for EBOV by RT-PCR (Xpert Ebola Assay, GeneXpert Instrument Systems; Cepheid, Sunnyvale, CA, USA) with a cycle threshold (C_t) of 32.5. Baby 2 tested negative for EBOV on day 1 and 72 hours later. Baby 2 was tested twice because he was considered at high risk for infection after being breast-fed for 6 days while his mother was symptomatic (i.e., until day 1 of her hospital admission).

On day 1, the woman was given convalescent-phase plasma from EBOV survivors; the treatment was given according to a compassionate-use protocol and was the standard process in this center at the time. On day 6, breast milk was sampled and tested positive for EBOV (C_t 21.6) (Table). The woman's clinical course was favorable; she remained afebrile during hospitalization, but mild symptoms persisted until day 5. The first convalescent-phase test, done on day 14, showed C_t values of 40.5 and 27.5 for blood and breast milk, respectively. On day 21, a second breast milk sample tested positive (C_t 32.7). On day 24, the woman was given cabergoline (0.5 mg 2×/d for 2 days) to cease lactation, after which no more breast milk samples could be collected. On day 29 after admission, she tested negative for EBOV in blood and urine and was reunited with baby 2. Serologic testing for baby 2 was done on day 23 and showed no sign of previous subclinical infection (ELISA, IgM, and IgG negative).

Many questions in this case remain unanswered, but our findings show the potential infectivity of breast milk for

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Technical Appendix

Porcine Deltacoronavirus Outbreak Investigation

During June–July 2015, the Center of Excellence for Emerging and Re-emerging Diseases in Animals at Chulalongkorn University (Bangkok, Thailand) investigated a suspected outbreak of porcine deltacoronavirus (PDCoV) infection in piglets with acute diarrhea, gilts, and sows that occurred on a commercial swine farm. Epidemiologic investigation, pathologic examination, sample collection, and laboratory diagnosis were conducted to determine the cause of the outbreak.

Identification of Porcine Deltacoronavirus

Blood ($n = 10$), intestine ($n = 8$), lymph node ($n = 2$), feces ($n = 6$), and feed ($n = 4$) samples were collected for 2 day-old piglets and 17-, 19-, and 20-week-old fattening pigs. Because sick pigs had clinical signs similar to those of pigs with other swine virus diseases, all samples were tested for transmissible gastroenteritis coronavirus; PEDV; rotaviruses A, B, and C; porcine reproductive and respiratory syndrome virus, and circovirus (1–5). For PDCoV identification, RNA was extracted from homogenized tissue and fecal, blood, and feed samples by using the QIAamp RNA Mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions.

cDNA was synthesized by using the Improm-II Reverse Transcription System and random primer (Promega, Madison, WI, USA). In brief, 5 μL of virus RNA and 5 μL of random primers (100 $\mu\text{mol/L}$) in a total reaction volume of 22 μL were incubated at 70°C for 15 min and at 4°C for 5 minutes. A total of 4 μL of 5 \times cDNA buffer, 1 μL of 0.5 mmol/L dNTP mixture, 2 μL of 2.5 mmol/L MgCl_2 , 0.3 μL of RNase inhibitor, 1 μL of ImProm-II reverse transcriptase, and 3.7 μL of distilled water were added to the RNA–primer mixture. The mixture was incubated at 25°C for 5 min, 42°C for 60 min, and 72°C for 15 min.

PDCoV identification was conducted by using a PCR protocol previously described (6). In brief, 10 μL of PCR mixture contained 0.5 μL of cDNA, 0.4 μL (10 $\mu\text{mol/L}$) of each forward and reverse primer, 5 μL of 2 \times TOP Taq Master Mix (QIAGEN), 1 μL of 10 \times CoralLoad, and 2.7 μL of distilled water. PCR conditions for PDCoV identification were initial denaturation at 94°C for 3 min;

40 cycles of denaturation at 94°C for 30 s, annealing at 50°C for 45 s, and extension at 72°C for 1 min; and final extension at 72°C for 7 min.

PCR products were then visualized by gel electrophoresis on a 1.2% of agarose gel in 0.5× Tris borate EDTA). Expected PDCoV product sizes were 500 bp for the membrane gene and 700 bp for the nucleocapsid gene.

Characterization of Porcine Deltacoronavirus

We selected 2 PDCoVs (S5011 and S5015L) for whole-genome sequencing and 14 PDCoVs for sequencing of spike, envelope, membrane, and nucleocapsid genes and the 3′-untranslated region. PDCoV genomes were amplified by using PCR and oligonucleotide primer sets previously described or new primer sets designed by using the Primer3 program (6–8). Primer sequences are available upon request.

A total of 30 µL of PCR mixture contained 2 µL of cDNA, 1.2 µL (10 µmol/L) of each forward and reverse primer, 15 µL of 2× TOPTaq Master Mix (QIAGEN), 3 µL of 10× CoralLoad, and 8.1 µL of distilled water. PCR conditions were initial denaturation at 94°C for 3 min; 40 cycles of denaturation at 94°C for 30 s, annealing at 48°C for 45 s, and extension at 72°C for 2 min; and final extension at 72°C for 7 min. Amplicons were gel-purified and sequenced (1st Base Laboratories, Kembangan, Malaysia).

Nucleotide sequences were assembled and validated by using SeqMan software version 5.03 (DNASTAR Inc., Madison, WI, USA). Nucleotide sequences of PDCoVs from Thailand were submitted to GenBank under accession nos. KU-51641–KU051656.

For pairwise comparison and genetic analysis of PDCoVs, nucleotide sequences and deduced amino acids of PDCoVs from Thailand were aligned with those of reference PDCoVs from China, South Korea, and the United States by MEGA version 6.06 and MegAlign version 5.03 (DNASTAR Inc., Madison, WI, USA) software. For phylogenetic analysis, whole-genome sequences of PDCoVs from Thailand were compared with those of reference PDCoVs. Phylogenetic analysis was performed by using MEGA version 6.06 (<http://www.megasoftware.net/>) with the neighbor-joining algorithm and bootstrap analysis of 1,000 replications. Additional analysis was performed by using BEAST software (<http://beast.bio.ed.ac.uk/>) and Bayesian Markov chain Monte Carlo methods with 5,000,000 generations and an average SD of split frequencies <0.05 (9–11).

References

1. Alfieri AA, Leite JP, Alfieri AF, Jiang B, Glass RI, Gentsch JR. Detection of field isolates of human and animal group C rotavirus by reverse transcription-polymerase chain reaction and digoxigenin-labeled oligonucleotide probes. *J Virol Methods*. 1999;83:35–43. [PubMed](#) [http://dx.doi.org/10.1016/S0166-0934\(99\)00104-4](http://dx.doi.org/10.1016/S0166-0934(99)00104-4)
2. Chen JF, Sun DB, Wang CB, Shi HY, Cui XC, Liu SW, et al. Molecular characterization and phylogenetic analysis of membrane protein genes of porcine epidemic diarrhea virus isolates in China. *Virus Genes*. 2008;36:355–64. [PubMed](#) <http://dx.doi.org/10.1007/s11262-007-0196-7>
3. Gouvea V, Allen JR, Glass RI, Fang ZY, Bremont M, Cohen J, et al. Detection of group B and C rotaviruses by polymerase chain reaction. *J Clin Microbiol*. 1991;29:519–23. [PubMed](#)
4. Nguyen VG, Moon HJ, Park SJ, Kim HK, Keum HO, Rho SM, et al. Multiplex PCR for PRRSV detection and typing. In: D’Allaire S, Friendship R, editors. 21st International Pig Veterinary Society Congress, July 18–21, 2010. Vancouver, British Columbia, Canada; 2010. Perry (IA): American Association of Swine Veterinarians [cited 2016 Jan 5].
https://www.aasv.org/library/swineinfo/series_index.php?id=9#82
5. Song DS, Kang BK, Oh JS, Ha GW, Yang JS, Moon HJ, et al. Multiplex reverse transcription-PCR for rapid differential detection of porcine epidemic diarrhea virus, transmissible gastroenteritis virus, and porcine group A rotavirus. *J Vet Diagn Invest*. 2006;18:278–81. [PubMed](#) <http://dx.doi.org/10.1177/104063870601800309>
6. Wang L, Byrum B, Zhang Y. Detection and genetic characterization of deltacoronavirus in pigs, Ohio, USA, 2014. *Emerg Infect Dis*. 2014;20:1227–30. [PubMed](#) <http://dx.doi.org/10.3201/eid2007.140296>
7. Koressaar T, Remm M. Enhancements and modifications of primer design program Primer3. *Bioinformatics*. 2007;23:1289–91. [PubMed](#) <http://dx.doi.org/10.1093/bioinformatics/btm091>
8. Untergasser A, Cutcutache I, Koressaar T, Ye J, Faircloth BC, Remm M, et al. Primer3: new capabilities and interfaces. *Nucleic Acids Res*. 2012;40:e115. [PubMed](#) <http://dx.doi.org/10.1093/nar/gks596>
9. Drummond AJ, Rambaut A. BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evol Biol*. 2007;7:214. [PubMed](#) <http://dx.doi.org/10.1186/1471-2148-7-214>
10. Drummond AJ, Suchard MA, Xie D, Rambaut A. Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Mol Biol Evol*. 2012;29:1969–73. [PubMed](#) <http://dx.doi.org/10.1093/molbev/mss075>

11. Tamura K, Dudley J, Nei M, Kumar S. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Mol Biol Evol.* 2007;24:1596–9. [PubMed](#)
<http://dx.doi.org/10.1093/molbev/msm092>
12. Woo PC, Lau SK, Lam CS, Lau CC, Tsang AK, Lau JH, et al. Discovery of seven novel mammalian and avian coronaviruses in the genus deltacoronavirus supports bat coronaviruses as the gene source of alphacoronavirus and betacoronavirus and avian coronaviruses as the gene source of gammacoronavirus and deltacoronavirus. *J Virol.* 2012;86:3995–4008. [PubMed](#)
<http://dx.doi.org/10.1128/JVI.06540-11>
13. Wang YW, Yue H, Fang W, Huang YW. Complete genome sequence of porcine deltacoronavirus strain CH/Sichuan/S27/2012 from mainland China. *Genome Announc.* 2015;3:e00945–15. [PubMed](#)
14. Song D, Zhou X, Peng Q, Chen Y, Zhang F, Huang T, et al. Newly emerged porcine deltacoronavirus associated with diarrhoea in swine in China: identification, prevalence and full-length genome sequence analysis. *Transbound Emerg Dis.* 2015;62:575–80. [PubMed](#)
15. Li G, Chen Q, Harmon KM, Yoon KJ, Schwartz KJ, Hoogland MJ, et al. Full-length genome sequence of porcine deltacoronavirus strain USA/IA/2014/8734. *Genome Announc.* 2014;2:e00278–14. [PubMed](#)
<http://dx.doi.org/10.1128/genomeA.00278-14>
16. Lee S, Lee C. Complete genome characterization of Korean porcine deltacoronavirus strain KOR/KNU14–04/2014. *Genome Announc.* 2014;2:e01191–14. [PubMed](#) <http://dx.doi.org/10.1128/genomeA.01191-14>

Technical Appendix Table 1. Characteristics of pig samples examined for porcine deltacoronavirus and other viruses, Thailand, 2015*

Sample ID	Date of collection	Pig age	Sample	PDCoV	PEDV	TGEV	RVA	RVB	RVC	PPRSV	Circovirus
S5011	Jun 10	2 d	Intestine	+	–	–	–	–	–	–	–
S5012	Jun 10	2 d	Intestine	+	–	–	–	–	–	–	–
S5013	Jun 10	NA	Feces	+	–	–	–	–	–	–	–
S5014P	Jun 30	2 d	GI pool	+	–	–	–	–	–	–	–
S5014J	Jun 30	2 d	Jejunum	+	–	–	–	–	–	–	–
S5014I	Jun 30	2 d	Ileum	+	–	–	–	–	–	–	–
S5014M	Jun 30	2 d	MLN	+	–	–	–	–	–	–	–
S5015P	Jun 15	2 d	GI pool	+	–	–	–	–	–	–	–
S5015J	Jun 30	2 d	Jejunum	+	–	–	–	–	–	–	–
S5015I	Jun 30	2 d	Ileum	+	–	–	–	–	–	–	–
S5015M	Jun 30	2 d	MLN	+	–	–	–	–	–	–	–
S5016	Jun 30	NA	Feces	+	–	–	–	–	–	–	–
S5017	Jun 30	2 d	Blood	+	–	–	–	–	–	–	–
S5018	Jun 30	2 d	Blood	+	–	–	–	–	–	–	–
S5019	Jun 30	2 d	Blood	+	–	–	–	–	–	–	–
S5020	Jun 30	2 d	Blood	+	–	–	–	–	–	–	–
S5021	Jun 30	2 d	Blood	+	–	–	–	–	–	–	–
F1	Jun 30	NA	Feed†	–	–	–	–	–	–	–	–
F2	Jul 13	NA	Feed‡	–	–	–	–	–	–	–	–
F3	Jul 13	NA	Feed‡	–	–	–	–	–	–	–	–
F4	Jul 13	NA	Feed‡	–	–	–	–	–	–	–	–
S5022	Jul 13	19 wk	Feces	+	–	–	–	–	–	–	–
S5023	Jul 13	19 wk	Feces	+	–	–	–	–	–	–	–
S5024	Jul 13	20 wk	Feces	+	–	–	–	–	–	–	–
S5025	Jul 13	20 wk	Feces	+	–	–	–	–	–	–	–
S5026	Jul 20	17 wk	Blood	+	–	–	–	–	–	–	–
S5027	Jul 20	17 wk	Blood	+	–	–	–	–	–	–	–
S5028	Jul 20	17 wk	Blood	+	–	–	–	–	–	–	–
S5029	Jul 20	17 wk	Blood	+	–	–	–	–	–	–	–
S5030	Jul 20	17 wk	Blood	+	–	–	–	–	–	–	–

*ID, identification; PDCoV, porcine delta coronavirus; PEDV, porcine epidemic diarrhea virus; TGEV, transmissible gastroenteritis coronavirus; RVA, rotavirus A; RVB, rotavirus B; RVC, rotavirus C; PPRSV, porcine reproductive and respiratory syndrome virus; +, p[ositive]; – negative; NA, not available; GI, gastrointestinal; MLN, mesenteric lymph node. References for primers in PCR: PDCoV (6); PEDV (2); TGEV and RVA (5); RVB (3); RVC (1); PPRSV (4); circovirus (Veterinary Diagnostic Laboratory, Chulalongkorn University).

†From a sow.

‡From a finishing pig.

Technical Appendix Table 2. Characterization of 16 porcine deltacoronaviruses Thailand, 2015*

Virus	Sample	Date of collection	Pig age	Gene sequenced	GenBank accession no.
PDCoV/Swine/Thailand/S5011/2015	Jejunum	Jun 10	2 d	Whole genome	KU051641
PDCoV/Swine/Thailand/S5012/2015	Jejunum	Jun 10	2 d	S, E, M, N	KU051642
PDCoV/Swine/Thailand/S5013/2015	Feces	Jun 10	2 d	S, E, M, N	KU051643
PDCoV/Swine/Thailand/S5014J/2015	Jejunum	Jun 30	2 d	S, E, M, N	KU051644
PDCoV/Swine/Thailand/S5014I/2015	Ileum	Jun 30	2 d	S, E, M, N	KU051645
PDCoV/Swine/Thailand/S5014L/2015	MLN	Jun 30	2 d	S, E, M, N	KU051646
PDCoV/Swine/Thailand/S5015J/2015	Jejunum	Jun 30	2 d	S, E, M, N	KU051647
PDCoV/Swine/Thailand/S5015I/2015	Ileum	Jun 30	2 d	S, E, M, N	KU051648
PDCoV/Swine/Thailand/S5015L/2015	MLN	Jun 30	2 d	Whole genome	KU051649
PDCoV/Swine/Thailand/S5016/2015	Feces	Jun 30	2 d	S, E, M, N	KU051650
PDCoV/Swine/Thailand/S5018/2015	Blood	Jun 30	2 d	S, E, M, N	KU051651
PDCoV/Swine/Thailand/S5019/2015	Blood	Jun 30	2 d	S, E, M, N	KU051652
PDCoV/Swine/Thailand/S5022/2015	Feces	Jul 13	19 wk	S, E, M, N	KU051653
PDCoV/Swine/Thailand/S5023/2015	Feces	Jul 13	19 wk	S, E, M, N	KU051654
PDCoV/Swine/Thailand/S5024/2015	Feces	Jul 13	20 wk	S, E, M, N	KU051655
PDCoV/Swine/Thailand/S5025/2015	Feces	Jul 13	20 wk	S, E, M, N	KU051656

*PDCoV, porcine deltacoronavirus; MLN, mesenteric lymph node; S, spike; E, envelope; M, membrane; N, nucleocapsid.

Technical Appendix Table 3. Pairwise comparison of nucleotides and amino acids of Thai/S5011 porcine deltacoronavirus with those of reference viruses, Thailand, 2015*

Viruses	Gene, nucleotide (amino acid) identities, %							
	Whole genome	ORF1ab, 18,804 bp	S, 3,480 bp	E, 252 bp	M, 654 bp	NS6, 285 bp	N, 1,029 bp	NS7, 603 bp
China, Hong Kong†	98.03–98.43	98.14–98.57 (98.45–98.89)	95.95–96.68 (97.38–98.17)	99.19–100 (100)	98.60–99.07 (99.54–100)	97.86–98.94 (97.85–100)	97.29–97.81 (98.82–99.41)	97.63–98.32 (93.81–95.40)
United States‡	98.10–98.12	98.22–98.25 (98.47–98.55)	95.93–96.10 (97.20–97.82)	99.19–99.60 (100)	98.29–98.44 (100)	98.22–98.58 (98.93)	96.88–97.09 (98.53–99.12)	97.27–97.62 (92.74–93.81)
South Korea§	98.10	98.23 (98.57)	96.10 (97.73)	99.60 (100)	98.28 (100)	98.58 (98.93)	96.99 (99.12)	97.46 (93.28)
Thailand¶	99.98	99.99 (99.97)	99.97–100 (99.91–100)	100 (100)	100 (100)	100 (100)	100 (100)	100 (100)

*Gene sizes for comparison are based on isolate HKU15–155. ORF, open reading frame; S, spike; E, envelope; M, membrane; NS, nonstructural; N, nucleocapsid.

†China/AH2004/2004, HKU/15–44/2009, HKU/15–155/2010, China/S27/2012, China/HB2014/2014, China/JS2014/2014, China/JXNI2/2015.

‡USA/IA8734/2014, USA/IL121/2014, USA/IL136/2014, USA/MI8977/2014, USA/OH137/2014, USA/OH1987/2014.

§KOR/KNU14–04/2014.

¶Thailand/S5015L/2015, Thailand/S5012/2015, Thailand/S5013/2015, Thailand/S5014J/2015, Thailand/S5014I/2015, Thailand/S5014L/2015, Thailand/S5015J2015, Thailand/S5015I2015, Thailand/S5016/2015, Thailand/S5018/2015, Thailand/S5019/2015, Thailand/S5022/2015, Thailand/S5023/2015, Thailand/S5024/2015, Thailand/S5025/2015.

Technical Appendix Table 4. Genetic analysis of nucleotide sequences of porcine deltacoronaviruses from Thailand and viruses from 3 other countries, 2015*

Country, virus	GenBank accession no.	Year isolated	Genome size, bp†	5'-UTR, 3-nt deletion at position	5'-UTR, 1-nt deletion at position	ORF1a, 6- nt deletion at position	ORF1a, 9- nt deletion at position	S gene, 3- nt insertion at position	3'-UTR, 3- or 4-nt insertion at position	3'-UTR, 1-nt deletion at position	Ref
				116–118	302	1737–1742	2808–2816	19473– 19474	25043– 25044	25258	
China											
HKU/15–44/2009	JQ065042	2009	25,421	No	No	No	No	ATT	GTT	T	(12)
HKU/15–155/2010‡	JQ065043	2010	25,416	No	No	No	No	No	No	No	(12)
China/S27/2012	KT266822	2012	25,404	No	No	AGTTTG	GAGCCAG TC	No	GTT	No	(13)
China/JXNI2/2015§	KR131621	2015	25,419	No	No	No	No	No	TT	No	(14)
United States											
USA/IA8734/2014¶	KJ567050	2014	25,422	No	No	No	No	AAT	GTT	No	(15)
South Korea											
KOR/KNU14–04/2014	KM820765	2014	25,422	No	No	No	No	AAT	GTT	No	(16)
Thailand											
Thailand/5011/2015#	NA	2015	25,404	TCT	A	AGTTTG	GAGCCAG TC	AAT	CTCT	No	This study
Thailand/5013/2015**	NA	2015	S, E, M, N genes	NA	NA	NA	NA	AAT	CTCT	NA	This study

*The reference virus was HKU15–155. UTR, untranslated region; ORF, open reading frame; Ref, reference; S, spike; E, envelope; M, membrane; N, nucleocapsid, NA, not available.

†Genome size does not include the polyA tail.

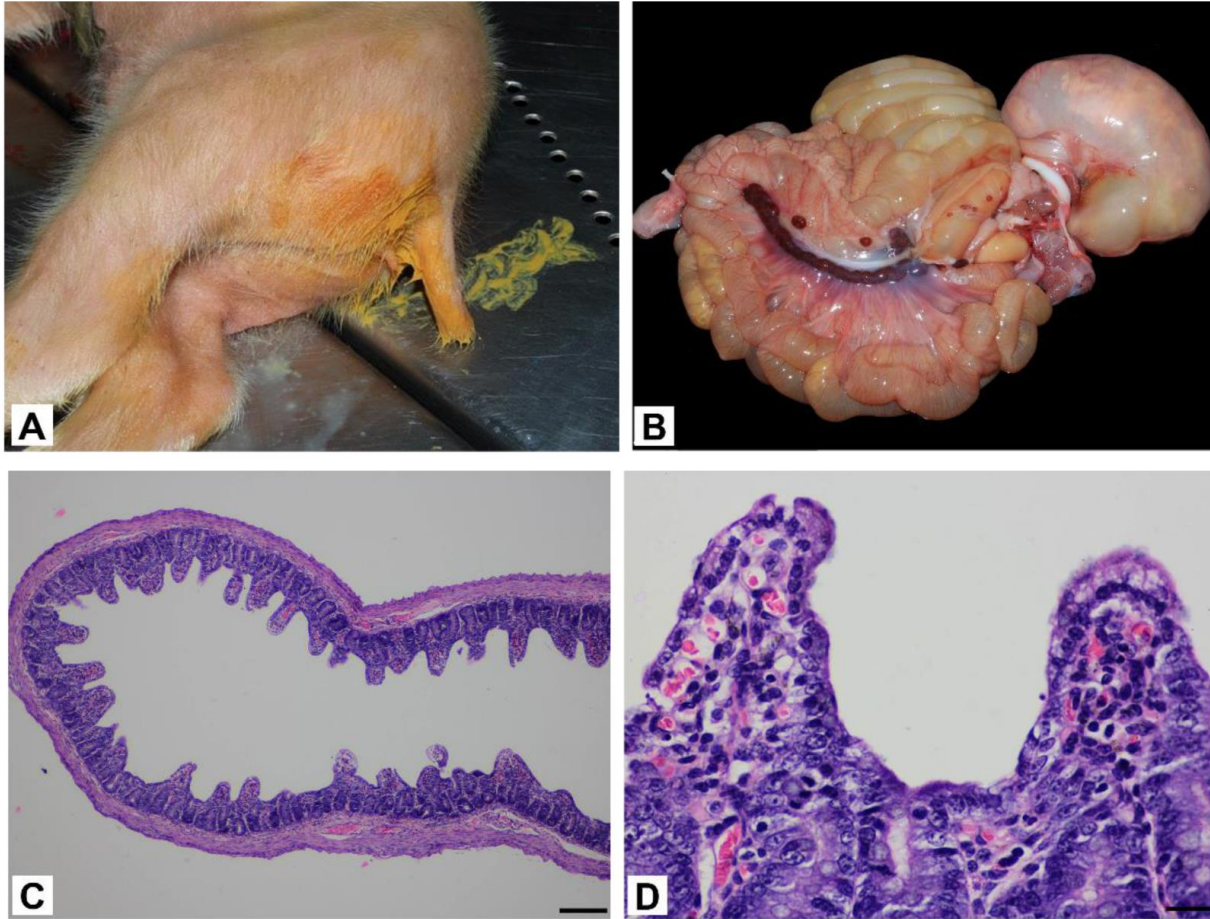
‡HKU/15–155/2010; China/AH2004/2004.

§China/JXNI2/2015, China/HB2014/2014, China/JS2014/2014.

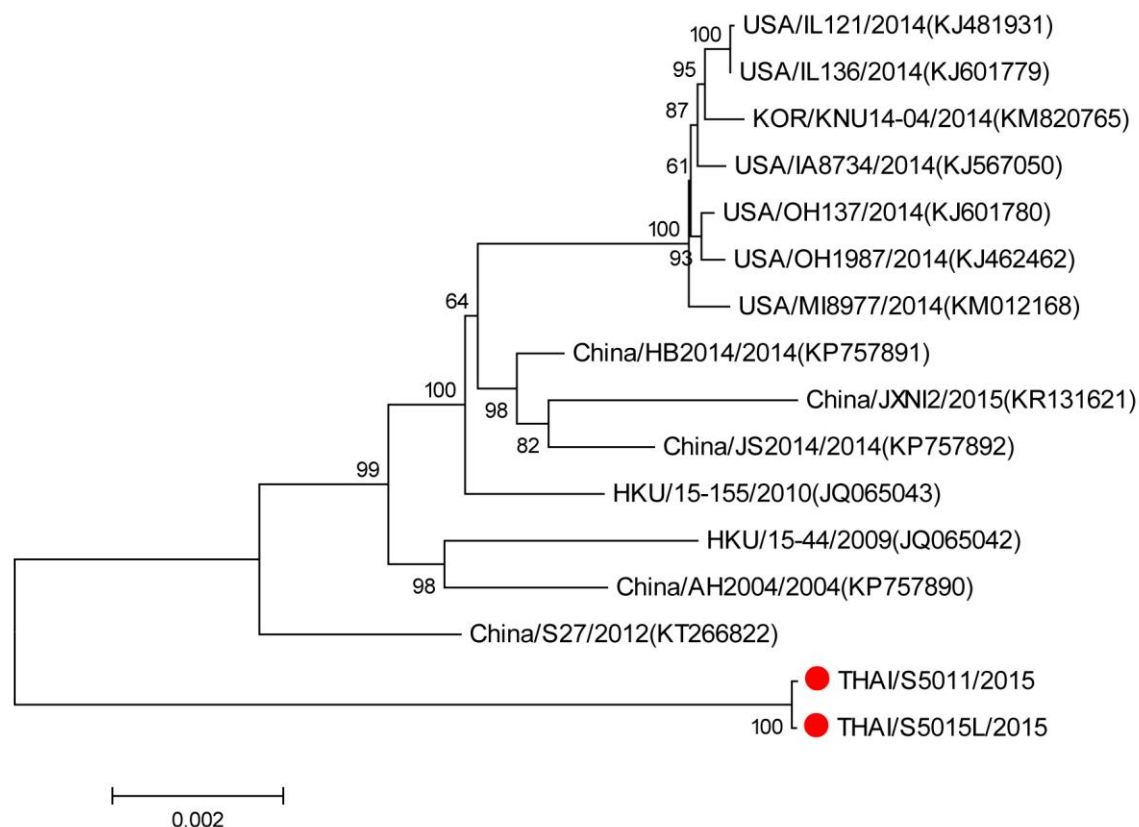
¶USA/IA8734/2014, USA/IL121/2014, USA/IL136/2014, USA/MI8977/2014, USA/OH137/2014, USA/OH1987/2014.

#Thailand/5011/2015, Thailand/5015L/2015, Thailand/5012/2015, Thailand/5014L/2015, Thailand/5022/2015.

**Thailand/5013/2015, Thailand/5014J/2015, Thailand/5014I/2015, Thailand/5015J2015, Thailand/5015I2015, Thailand/5016/2015, Thailand/5018/2015, Thailand/5019/2015, Thailand/5023/2015, Thailand/5024/2015, Thailand/5025/2015.



Technical Appendix Figure 1. Analysis of pigs infected with porcine deltacoronavirus, Thailand, 2015. A) Gross findings of emaciated piglet showing yellow pasty feces. B) Curdled milk in gastric lumen and thin intestinal wall containing watery content and curdled milk. Milk veins were absent. C) Histopathologic analysis showing shortened and occasionally fused villi. Scale bar = 400 μm . D) Histopathologic analysis showing attenuated and vacuolated cytoplasm of enterocytes. Scale bar = 40 μm .



Technical Appendix Figure 2. Phylogenetic analysis open reading frame 1a/b of porcine deltacoronaviruses, Thailand, 2015. Red circles indicate strains isolated in this study. The tree was constructed by using MEGA version 6.06 program (<http://www.megasoftware.net/>) with the neighbor-joining algorithm and bootstrap analysis with 1,000 replications. Numbers along branches are bootstrap values. Scale bar indicates nucleotide substitutions per site.